

TINF/TNFR Working Party Distribution List for October 18, 1995 Team Meeting

SB
Edward R Appelbaum
M. Burke
E. Dul
Edward R Gimmi
Don E Griswold
Jeremy A Harrop
Yen Sea Ho-1
Mark R Hurle
John C Lee
Sally D Lyn
Gordon P Moore
Terence Porter
Manjula P Reddy
Carol Silverman
Kong B Tan
Ping Tsui-1
X. Wang
Peter R Young
T. L. Yue

SB cc: List
Sherin S Abdel-Meguid
Christine M Debouck
Giora Z Feuerstein
George I Glover
Maxine Gowen
C Preston Hensley
Zdenka L Jonak
Kristy Kikly-1
Loretta C McCauley
Jo Anne Mackey
Andrew J Nichols
Martin Rosenberg
Allan R Shatzman
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HGS
Reiner Gentz
Jian Ni
Guo-Liang Yu
Susan Lackey

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Ruben EXHIBIT 2071
Ruben v. Wiley et al.
Interference No. 105,077
RX 2071

TNF/TNFR Working Party: Minutes of October 18, 1995 SB/HGS Joint Meeting
Meeting held at Upper Merion on October 18, 1995.

Attendee:

SB: E. Appelbaum, M. Burke, E. Dul, J. Harrop, M. Hurle, Z. Jonak, K. Krikly, J. Lee, S. Lyn, G. Moore, M. Reddy, K.B. Tan, P. Tsui, A. Truneh, P. Young, X. Wang, T.L. Yue

HGS: R. Gentz, J. Ni, G-L. Yu,

P. Young presented a critical path depicting key components a strategy for identification of gene function for TNF/TNFR related genes (overhead PY1).

M. Hurle reviewed the sequences of 8 novel TNFR like and 3 TNF like genes (overheads MH1-6).

J. Ni reviewed for the group the current members of known TNF and TNFR superfamily, as well as the recently described associated proteins. Many of the known proteins are present in the HGS database as full length genes. The rat Fas ligand like molecule has been expressed in *E. coli* and polyclonal antibody is generated. The second TNF like ligand, termed TNF γ or EDAP, is being pursued by HGS as a novel antiviral agent. This molecule has been expressed and purified at HGS. The third TNF like ligand has been shown to be expressed in most tissues with the highest expression in the heart. Of the eight novel TNFR like proteins, one appears to be a soluble receptor to which antibody has been made. HGS is more interested in working on the ligands than the receptors.

K.B. Tan received the tissue/cell distribution of the 5 most interesting TNF/TNFR genes. The results are summarized in overheads KB1-8. Most of these genes (TR2, TR3 and TL1) appear to be primarily expressed in hematopoietic cells while TR1 message was found in an osteoclast cell line. A larger size message for TR1 was also found in a hematopoietic precursor and a T cell line.

P. Young/S. Lynn provided an update on the efforts of cloning and expressing what is now known as TR2. Expression of the human IgG1 Fc-fusion protein in mammalian cells is successful. Monoclonal antibody production has been initiated.

E. Applebaum reported the expression of the rat Fas ligand homolog in two fusion constructs as epitope tagged proteins.

A number of issues surfaced during the ensuing discussion period and they are listed below with solutions and/or action plans:

1. *Nomenclature*

Given that there are multiple designations of the original ESTs by HGS and subsequent names/numbers given by SB/ATG and the increasing number of interesting molecules, it has become apparent that an unified nomenclature should be established. It was agreed all the novel TNF/TNFR related full length genes will have T as the first prefix to denote their homology to these superfamilies, followed by L or R to denote membership to the ligand or receptor superfamily, followed by a unique number for each unique full length novel sequence. Splice variants will be sub-classified with an alphabet; e.g. TR2a for HLHAB49 (see also post meeting Tables 1 and 2 in AT1-2 for details).

2. *Focus*

HGS will focus on putative ligands and SB on the receptors

3. *Sequence errors/differences between HGS and SB*

Consultation among the two parties is recommended before any work is to be done. Any discrepancies in sequence that may have impact on patents must seek legal input.

4. *Spliced variants*

Whether spliced variants are meaningful targets would require further confirmation by PCR of the inserts and TNT tests should be conducted.

5. *Reagent for exchange*

Currently, the following tool reagents are available:
SB: TR2-Ig
HGS: TL1 and TR1 proteins and antisera to TL2 and TR1

6. *Coordination of expression efforts*

SB will concentrate on expressing all TR molecules and some TL proteins
HGS will express all TL proteins

7. *Third party MTA*

Established through HGS:

a. Alan Porter (U. Singapore) on TR2 (signal transduction) and TRAP clones (2 hybrid screen)

b. Kwon (Indiana U.) on TR2 (genomic cloning and spliced variant)

Pending at HGS:

a. Aggarwal (MD Anderson) on TR1, TL1 etc (biological effects, functions and effect on tumor cells)

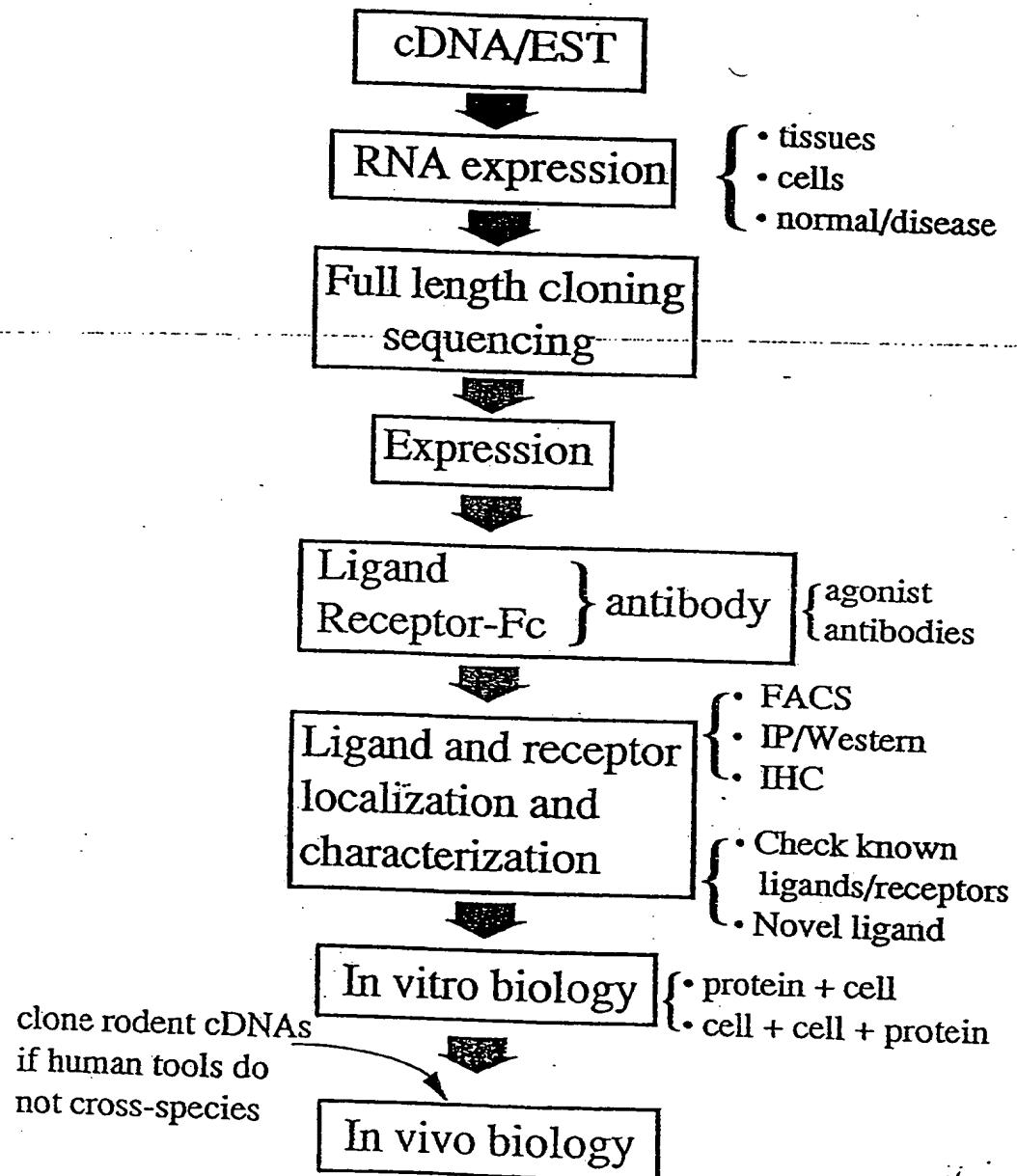
b. Dormer (Indiana U.) on novel TRAP molecules

Underdiscussion at SB:

a. Beutler (UTSW) on novel TRs (signaling mechanisms)

b. Kollias (Hellenic Pasteur institute) on novel TIs (transgenic/knockout)

Critical path



Novel TNF Receptor Homologs

Molecule	Project ID	#Clones	#ESTs	Homolog	% Identity	Length
TR1	HSABH13	5	11	human TNFR-II	28%	402aa
TR2	HLHAB49	15	58	mouse CD40	29%	284aa
TR2a	HHFFT79	3	18	(splice of TR2)		
TR2b	HMSBZ59	2	17	(splice of TR2)		
TR2c	HROAG75	1	2	(splice of TR2)		
TR3	HTTBH61	3	5	human TNFR-I	37%	71aa (extracellular)
TR4	HSAVO45	1	3	mouse TNFR-I	48%	79aa (death domain)
TR5	HTXEI33	1	4	human 4-1BB	100%	255aa (splice)

Other non-full length clones (class 2) in ATG/HGS database not listed above*

TNFR I	2
TNFR II	1
OX40	2
Fas	2
CD40	4
CD27	1
CrmB	1
4-1BB	1

* sequence analysis in progress

Novel TNF (Ligand) Homologs

Molecule	Project ID	#Clones	#ESTs	Homolog	% Identity	Length
TL1	HUVE091	4	29	human TNF α	30%	216aa
TL2	HTPAN08	17	36	rat Fas ligand	27%	125aa
TL3	HLTB771	12	28	rat TNF α	27%	120aa
TL4	HPDD012					

Other non-full length clones (class 2) in ATG/HGS database not listed above*

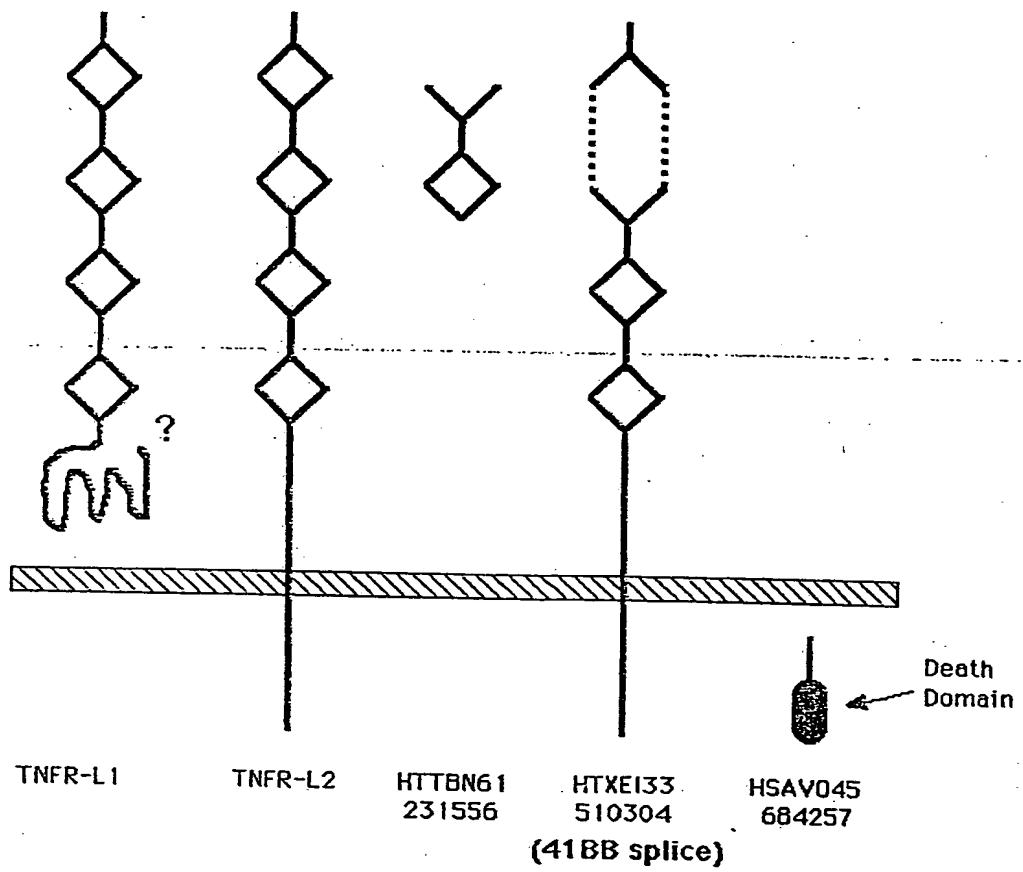
LT- α	2
LT- β	1
Fas Ligand	3
CD30 Ligand	2

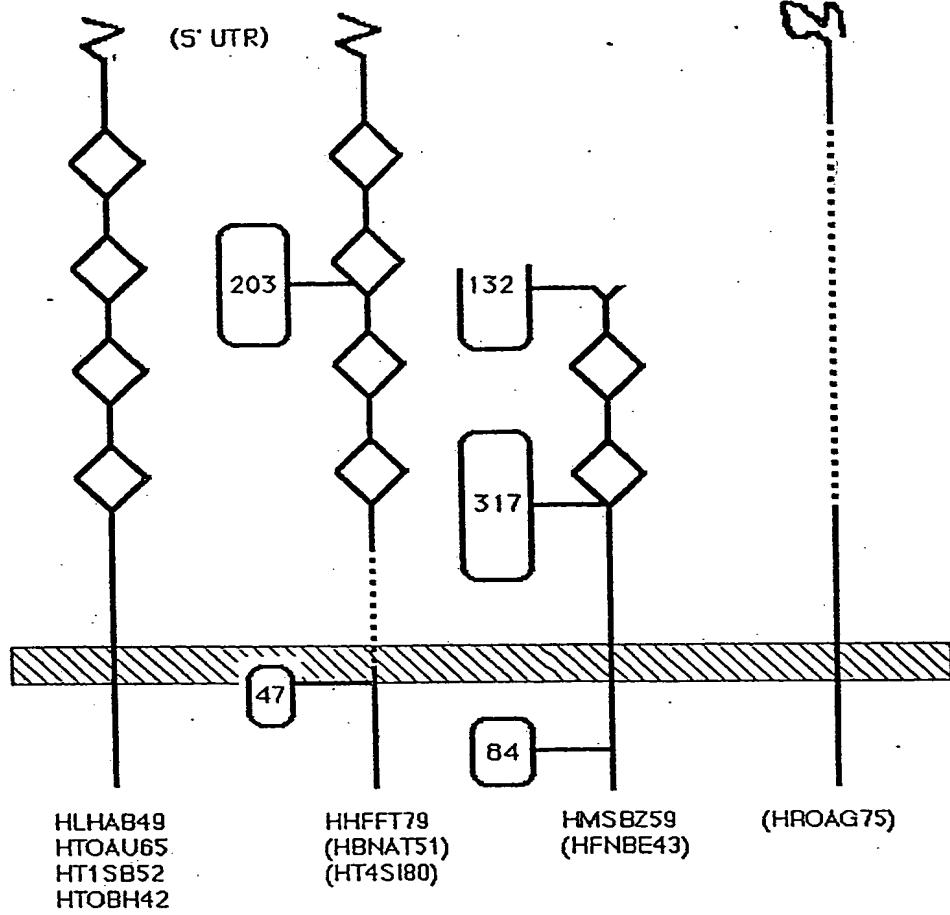
* sequence analysis in progress

Novel TNF Receptors and Ligands

Molecule	Project ID	#Clones	#ESTs	Homolog	% Identity	Length
TNFR-L1	HSABH13	5	11	human TNFR-2	28%	402aa
TNFR-L2	HLHAB49	15	58	mouse CD40	29%	284aa
TNFR-L2a	HHFFT79	3	18	(splice of TNFR-L2)		
TNFR-L2b	HMSBZ59	2	17	(splice of TNFR-L2)		
TNFR-L2c	HROAG75	1	2	(splice of TNFR-L2)		
TNFR-L3	HTTBPN61	3	5	human TNFR-1	37%	71aa (extracellular)
TNFR-L4	HSAVO45	1	3	mouse TNFR-1	48%	79aa (death domain)
41BB-L1	HTXE133	1	4	human 4-1BB	100%	255aa (splice)
TNF	HUVEO91	4	29	human TNF α	30%	216aa
TNF	HTPAN08	17	36	rat Fas ligand	27%	125aa
TNF	HLTBT71	12	28	rat TNF α	27%	120aa

Novel TNFR Receptor-Related Molecules





All solid lines have sequence in common

Sizes of splice inserts are shown inside oval-shaped symbol

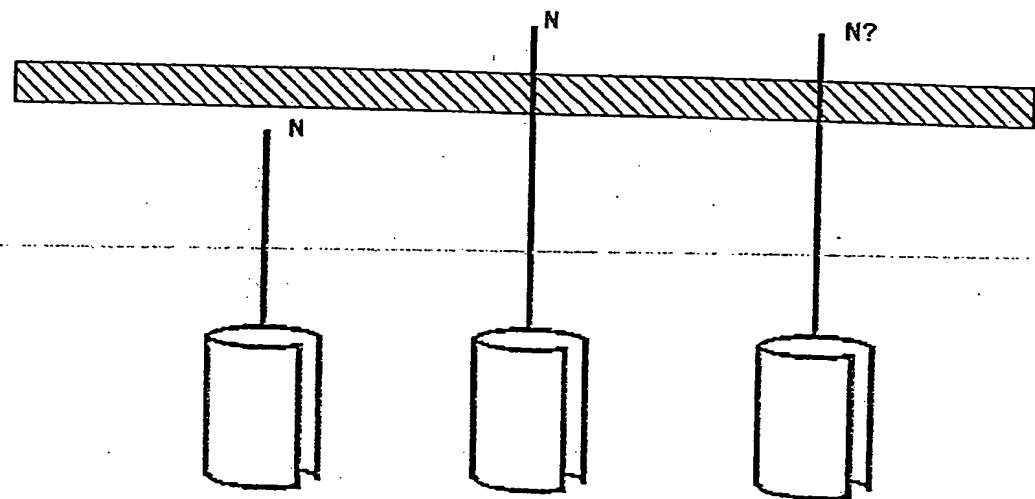
Project IDs for full length sequences or (individual ESTs) are shown

Novel TNF-Related Molecules

HUVE091
355074

HTPAN08
413412

HLTB71
875848



Summary of Receptor Expression

- Sequenced HTOAU65 (103902)
 - Named this TNFRL2
 - TNFRL2 differs from patent
 - Position 41 (Ser to Phe)
 - Position 17 is Arg (Lys in some ESTs)
 - one silent change
- Cloned into mammalian expression vector COSFc
 - Re-engineered start codon
- Expressed Human IgG1 Fc-fusion TNFRL2
 - COS cells
 - CHO-E1a
- Monoclonal antibody production initiated
- Sequenced HSABH13(195197)
 - Named this TNFRL1
 - Sequence different from patent
 - C 1077 missing
 - Altered C-terminus and an 11 aa longer protein

325532 TCTAGTTCTC TGCTGGAGTT CATCCCTGCTA GCTGGGGTTCG CGAGCTGCCG
 103902
 Tnfrl2fctextcontig_1 CTATATAAGC AGAGGCTGGT ACGTGACCCG TCAGATCGCC TGGAGACGCC

251
 338804 ATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG
 338805 GCTCTGAGCT GAGGCATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG
 Tnfrl2 CCT GAGGCATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG
 355073 CCT GAGGCATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG
 325531 GCTCTGAGCT GAGGCATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG
 325532 GCTCTGAGCT GAGGCATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG
 103902
 Tnfrl2fctextcontig_1 ATCGAATTAG CAGGCCATGGA GCCTCTCTGGA GACTGGGGGC CTCTCTCCCTG

301 Lys
 338804 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT
 338805 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT
 Tnfrl2 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT
 355073 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT
 325531 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT
 325532 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT
 103902
 Tnfrl2fctextcontig_1 GAGATCCACCC CCCCAGAACCG ACGTCTTGAG GCTGGTGCTG TATCTCACCT

351
 338804 TCCCTGGGAGC CCCCCTGCTAC GCCCCAGCTC TGCCGCTCTG CAAGGAGGAC
 338805 TCCCTGGGAGC CCCCCTGCTAC GCCCCAGCTC TGCCGCTCTG CAAGGAGGAC
 Tnfrl2 TCCCTGGGAGC CCCCCTGCTAC GCCCCAGCTC TGCCGCTCTG CAAGGAGGAC
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 325532 TCCCTGGGAGC CCCCCTGCTAC GCCCCAGCTC TGCCGCTCTG CAAGGAGGAC
 103902
 Tnfrl2fctextcontig_1 TCCCTGGGAGC CCCCCTGCTAC GCCCCAGCTC TGCCGCTCTG CAAGGAGGAC

401
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 338805 GAGTACCCAG TGGGCTCCGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG
 Tnfrl2 GAGTACCCAG TGGGCTCCGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG
 355073 GAGTACCCAG TGGGCTCCGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG
 325531 GAGTACCCAG TGGGCTCCGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG
 325532 GAGTACCCAG TGGGCTCCGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG
 103902 AATTG CCCGGGNAGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG
 Tnfrl2fctextcontig_1 GAGTACCCAG TGGGCTCCGA GTGCTGGCCC AAGTGCAGTC CAGGTTATCG

451
 338804 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 338805 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 Tnfrl2 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 355073 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 325531 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 325532 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 103902 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC
 Tnfrl2fctextcontig_1 TGTTGAAGGAG GCCTGGGGGG AGCTGACGGG CACAGTGTGT GAAACCTGCC

501
 338804 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGCTGCAG
 338805 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGCTGCAG
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 355073 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGCTGCAG
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 325532 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGCTGCAG
 103902 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGCTGCAG
 Tnfrl2fctextcontig_1 CTCCAGGCAC CTACATTGCC CACCTCAATG GCCTAAGCAA GTGCTGCAG

551
 338604 TCCCAATTGT GTGACCCAGC CATGGGGCTG CGGGGGAGCC GGAACTGCTC

Sally Lyle

GAP of: Tnfrl2.Pep che 4189 from: 1 to: 283

TRANSLATE of: tnfrl2.seq check: 9389 from: 9 to: 857
generated symbols 1 to: 283.

TNF receptor like 2 sequence was copied from HGS full length report

filename 103902.000, HGS code 103902, Project ID: HT1SB52

REFORMAT of: Tnfr2.Seq check: -1 from: 1 to: 881 May 16, 1995 13:54
(No documentation) ..

to: Tnfrl2fclink.Pep check: 9984 from: 1 to: 432

TRANSLATE of: tnfrl2fclink.seq check: 768 from: 665 to: 1960
generated symbols 1 to: 432.

Tnfrl2fclink is sequenced 103902fc (EcoRI Asp718 of
Tnfrl2fcContig1.seq) in Cosfclink.

COSfclink is SFcrlcos4 with the hinge CH2CH3 region of human IgG
inserted in place of the FcR cDNA and a 27bp linker inserted upstream ..

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Nwsgapppep.Cmp
CompCheck: 1254

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Length Weight: 0.100 Average Mismatch: -0.396

Quality: 315.8 Length: 439

Ratio: 1.116 Gaps: 6

Percent Similarity: 85.145 Percent Identity: 76.812

Tnfrl2.Pep x Tnfrl2fclink.Pep August 11, 1995 10:46 ..

1 MEPPGDWGPPPWSTPRTDVLRLVLYLTFLGAPCYAPALESKEDEYPVG 50

58 → 1 MEPPGDWGPPPWSTPRTDVLRLVLYLTFLGAPCYAPALESKEDEYPVG 50

2nd G:

51 S2CCPKCSPGVRKEACGELTGTVC~~EP~~PC~~PP~~GTYIAHNL~~S~~KCLQCMCD 100

51 SECCPKCSPGVRKEACGELTGTVC~~EP~~PC~~PP~~GTYIAHNL~~S~~KCLQCMCD 100

101 PAMGLRASRNC~~RT~~ENAVCGCSPG~~EF~~CTIVQDGDRCAACRAYATSSPGQRV 150

101 PAMGLRASRNC~~RT~~ENAVCGCSPG~~EF~~CTIVQDGDRCAACRAYATSSPGQRV 150

151 QKGGTESQDTLCQNC~~PP~~GTFSPNGTLEECQHOTKCSWLVTKA...GAGT 196

151 QKGGTESQDTLCQNC~~PP~~GTFSPNGTLEECQHOTKCSWLVTKARSIEGRGT 200

197 S.....SSRNWMFLSGSLVIVIVCSTVGLIICV.. 225

201 EPKSA~~DKT~~TTCPPCPAPELLGGPSVFLFPPKPKDTLMSRTPEVTCVVVD 250

225KRRKPRGD.....VVKVIVSVQRKRQE 247

251 VSHEDPEVKENWYDGVEVHNAKTRPREEQVNSTYRVVSVLTVLHQDWLN 300

242 AEGEATVI..EALQAPPDVTTVAVEETIPSFTGRSPNH..... 263

361 GKEYKCKVSENKALPAP.....IEKTISKARGQPREPQVYTLPPSPDEL 343

Sally Lyn

PY4

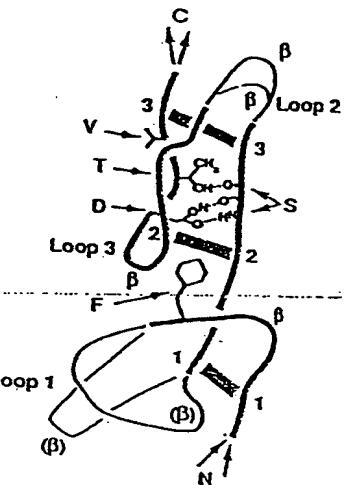
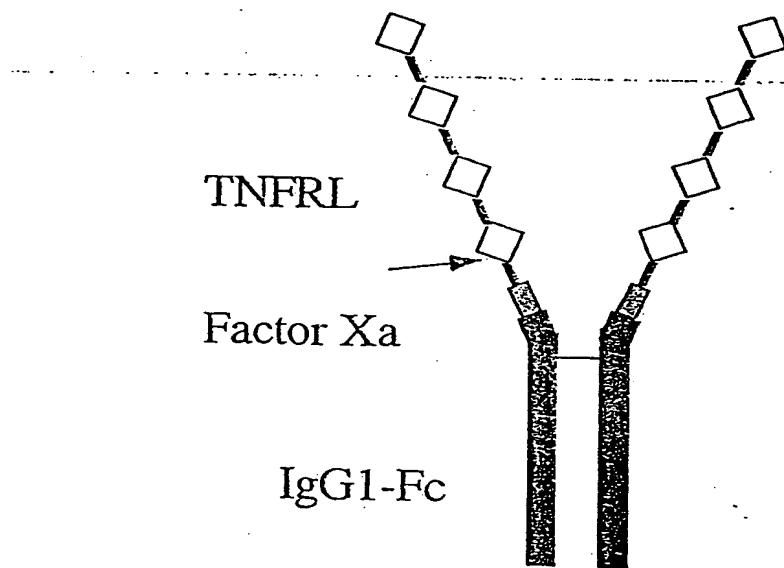


Figure 6. Schematic Representation of the sTNF-RSS Domain Fold (See also Figures 4 and 5.) The three disulfide bridges are represented by very thick lines and the structurally conserved parts by thick lines. (Here residues 55-60, 70-76, and 83-97 of domain 2 are taken as conserved; these superpose on domain 3 with an RMS error of 0.98 Å.) In the nonconserved regions, the domain 2 structure is shown as a medium line and the domain 3 structure as a thin line. The N- and C-terminal domains are marked N and C, respectively; the arrows are a reminder that the interdomain connections are not conserved. The second up-strand from loop 3 to the C-terminus carries the conserved Asp/Asn-Thr-Val motif, indicated by D, T, and V, and runs behind the down-strand. The conserved Ser is marked S, and the three strand-bridging hydrogen bonds it makes to D and T are shown as dotted lines. A β meander lies in front of the V and the third disulfide bridge. The conserved Tyr/Ph is indicated by F. It reaches from loop 1 behind the second disulfide bridge and packs against the conserved Asp/Asn to Ser bridge (D=S). The β turn positions are indicated by B.

Receptor-IgFc fusions



DV7

COOMASSIE STAINED SDS-PAGE GEL
ANALYSIS OF 8UG TNFRL2Fc UNDER
REDUCING AND NON-REDUCING
CONDITIONS

Non
Reduced Reduced

200D -	200KD -
93D -	93KD -
69D -	69KD -
46D -	46KD -
30KD -	30KD -
22KD -	22KD -

BESTFIT of: Stnfr11.Seq check: 8473 from: 1 to: 1173

REFORMAT of: Stnfrlike1.Seq check: -1 from: 1 to: 1173 May 9, 1995 10:28
Coding region of soluble TNFR pulled from ATG full length report
80245.000, project ID: HSABH13 (full length clone = 195197)

to: 195197contigedtext.Seq check: 4750 from: 1 to: 1850

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CompCheck: 5234

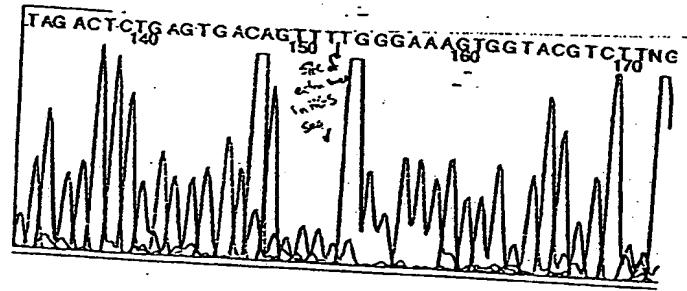
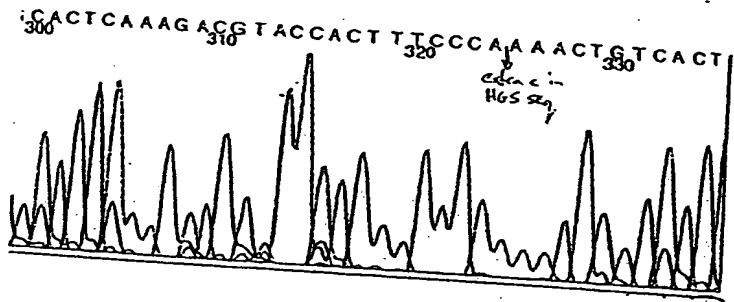
Gap Weight: 5.000 Average Match: -1.000
Length Weight: 0.300 Average Mismatch: -0.900

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Ratio: 0.995 Gaps: 1
Percent Similarity: 100.000 Percent Identity: 100.000

Stnfr11.Seq x 195197contigedtext.Seq October 17, 1995 16:21 ..

1951 GGCATGCAAACCCAGTGACCAAGATCCAGCTGCTCAGTTTGTCGGCGAA 1000
1270 GGCATGCAAACCCAGTGACCAAGATCCAGCTGCTCAGTTTGTCGGCGAA 1319
1001 TAAARARTGGCGACCAAGACACCTTGAGGGCTAATGCACGCACTAAAG 1050
1320 TAAARARTGGCGACCAAGACACCTTGAGGGCTAATGCACGCACTAAAG 1369
1051 CACTCAAAGACGTACCACTTCCCCAAGACTGTCACTCAGAGTCCTAAAGA 1100
1370 CACTCAAAGACGTACCACTTCCCCAAGACTGTCACTCAGAGTCCTAAAGA 1418
1101 AGACCATCAGGTTCCTCACAGCTTCACAAATGTACAAATTGTATCAGAAG 1150
1419 AGACCATCAGGTTCCTCACAGCTTCACAAATGTACAAATTGTATCAGAAG 1468
1151 TTATTTTAAAGAATGATAAGTAA 1173
1469 TTATTTTAAAGAATGATAAGTAA 1491

010



TRANSLATE of: stnfrlike1.seq check: 8473 from: 1 to: 1173
generated symbols 1 to: 391.

REFORMAT of: Stnfrlike1.Seq check: -1 from: 1 to: 1173 May 9, 1995 10:28
(No documentation)

Stnfrlike1.pep Length: 391 May 9, 1995 16:13 Type: P Check: 8773 ..

ATENY 1 MNKLLCCALV FLDISIKWTT QETFPKYLH YDEETSHQLL CDKCPPGTYL
51 EKQHCTAKWKT VCAPCPDHYY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT
101 HNRVCECKEG RYLEIEFCLK HRSCPFGFV VQAGTPERNT VCKRCPDGFF
151 SNETSSKAPC RKHINCSVFG LLLTQKGNAT HDNICSGNSE STQKCGIDVT
201 LCEEEAFFRFA VPTKFTPWL SVLVDNLPGT KVNAESVERI KRQHSSQEQT
251 FQLLKLWKHQ NKDQDIVKKI IQDIDLCENS VQFHIGHANL TFEQLRSLME
301 SLPGKKVGAE DIERTIKACK PSDQILKLLS IWRKNGDQD TLKGLMHALK
351 HSKTYHFPKT VTSQSLKKTIR FLHSFTIMYKL YQELFLEKIG NQVQSVKLSC

TRANSLATE of: 195197contigedtext.seq check: 4750 from: 320 to: 1525
generated symbols 1 to: 402.

195197contigedtext.Pep Length: 402 October 17, 1995 17:01 Type: P Check: 5187 ..

3CLONC 1 MNKLLCCALV FLDISIKWTT QETFPKYLH YDEETSHQLL CDKCPPGTYL
51 EKQHCTAKWKT VCAPCPDHYY TDSWHTSDEC LYCSPVCKEL QYVKQECNRT
101 HNRVCECKEG RYLEIEFCLK HRSCPFGFV VQAGTPERNT VCKRCPDGFF
151 SNETSSKAPC RKHINCSVFG LLLTQKGNAT HDNICSGNSE STQKCGIDVT
201 LCEEEAFFRFA VPTKFTPWL SVLVDNLPGT KVNAESVERI KRQHSSQEQT
251 FQLLKLWKHQ NKDQDIVKKI IQDIDLCENS VQFHIGHANL TFEQLRSLME
301 SLPGKKVGAE DIERTIKACK PSDQILKLLS IWRKNGDQD TLKGLMHALK
351 HSKTYHFPKT VTSQSLKKTIR FLHSFTIMYKL YQELFLEKIG NQVQSVKLSC
401 L*

PY10

BESTFIT of: Stnfr11.Pep check: 6773 from: 1 to: 391
TRANSLATE of: stnfrlikel.seq check: 8473 from: 1 to: 1173
generated symbols 1 to: 391.
REFORMAT of: Stnfrlikel.Seq check: -1 from: 1 to: 1173 May 9, 1995 10:28
(No documentation)

to: 195197contigedtext.Pep check: 5187 from: 1 to: 402

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generated symbols 1 to: 402.

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Length Weight: 0.100 Average Mismatch: -0.396

Quality: 543.8 Length: 400
Ratio: 1.424 Gaps: 1
Percent Similarity: 95.812 Percent Identity: 94.764

Stnfr11.Pep.x 195197contigedtext.Pep.October 17, 1995 17:02

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39 CLEVE 1 MNKLLCCALVFLDISIKWTTQETFPKYLHYDEETSHQLLCDKCPGTYL 50
51 KQHCTAKWKIVCAPCPDHYTDSWHTSDECILYCSPPVCKELOYVKQECNRT 100
51 KQHCTAKWKIVCAPCPDHYTDSWHTSDECILYCSPPVCKELOYVKQECNRT 100
101 HNRVCECKEGRYLEIEPCLKHRSCPPGFVQAGTPERNITVCKRCPDGFF 150
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151 SNETSSKAPCRKHTNCVFGLLLTKGNATHDNICSGNSESTQKCGIDVT 200
151 SNETSSKAPCRKHTNCVFGLLLTKGNATHDNICSGNSESTQKCGIDVT 200
201 LCEEEAFFRFRAVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSSQEQT 250
201 LCEEEAFFRFRAVPTKFTPNWLSVLVDNLPGTKVNAESVERIKRQHSSQEQT 250
251 FQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME 300
251 FQLLKLWKHQNKDQDIVKKIIQDIDLCENSVQRHIGHANLTFEQLRSLME 300
301 SLPGKKVGAEDIETKTIACKPSDQILKLLSLWRIKNGDQDTLKGLMHALK 350
301 SLPGKKVGAEDIETKTIACKPSDQILKLLSLWRIKNGDQDTLKGLMHALK 350
351 HSKTYHFPTRCHSESKEDHQVPSQLHNQIVS 382
351 HSKTYHFPKTVTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISC 400

Ligands

1. Fas ligand-like

HTPAN08

I, Protein was purified from E. coli. expression system.

II, Polyclonal antibody was made.

III, Expression and purification of protein from baculovirus is in progress.

VI, Expression in COS and CHO cells is in progress.

V, Functional study is in progress.

Ligands

2. TNF-like (TNF gamma)

HUVEO91

I, Two new constructs with different N-terminal sequences (no tag) were made, and expressed in E. coli. Protein was purified.

II, Functional Study is in progress.

III, Polyclonal and monoclonal antibodies are not available at present.

VI, Expression in COS and CHO cells is in progress.

V, Expression level of protein in baculovirus is low.

TNF Ligand, Receptor and Receptor Associated Factor Superfamily

I, Overview

**II, Class I TNF Ligand, Receptor and Receptor Associated
Factor Superfamily Members in HGS Database**

**III, Novel TNF Ligand, Receptor and Receptor Associated
Factor Superfamily Members in HGS Database**

<u>Ligands</u>	<u>Receptors</u>	<u>Receptor Associated Factors</u>
1, TNF α	TNFR1	FADD (MORT1), TRADD, TRAP-1, TRAP-2, TRAK, RIP,
2, LT α	TNFR2	EB16 (TRAF1), TRAF2 (TRAP-3), TRAF3 (CD40bp, LAP-1, CRAF-1, CAP-1)
3, LT β	TNFR-RP (LT β R)	EB16 (TRAF1)
4, FasL	Fas (APO-1)	FADD (MORT1), TRADD, RIP, FAP-1, FAST
5, CD40L	CD40	TRAF3 (CD40bp, LAP-1, CRAF-1, CAP-1) TRAF2 (TRAP-3), EB16 (TRAF1)
6, CD30L	CD30	??
7, CD27L	CD27	??
8, 4-1BBL	4-1BB	??
9, OX40L	OX40	??
10,	NGFR	
11,	PV-T2 (MyxomaT2)	??
12,	PV-A53R	??
13,	ECP1	??
14,	Cowpox CrmB	??
15,	VVsalf19R	??
16,	SFVT2	??
17,	G4R	??
18,	C22/B28R	??
19,	G2R	??

TNF Signal transducers

I, Receptor Associated Factors:

- 1, EB16 (TRAF1),**
- 2, TRAP-3(TRAF2),**
- 3, TRAF3 (CD40bp, LAP-1, CRAF-1, CAP-1, TRAF2)**
- 4, FADD (MORT1),**
- 5, TRADD,**
- 6, TRAP-1,**
- 7, TRAP-2,**
- 8, RIP**

II, Protein Tyrosine Phosphatases :

FAP-1

III, Protein Tyrosine Kinases:

- 1, TRAK**
- 2, FAST**

Class I TNF Ligand, Receptor and
Receptor Associated Factor Superfamily
Members in HGS Database

Ligands

1, TNF α	Yes, Full length,	HMPAD01
2, LT α	Yes, Full length,	HTAAU17
3, LT β	Yes, Full length,	HNHCT93
4, FasL	Yes, Full length,	HNFC016
5, CD40L	Yes, -320bp,	HLQAV55R
6, CD30L	Yes, -1000bp,	HSAVS23
7, CD27L	Yes, Full length,	HRGSD45
8, 4-1BBL	??,	??
9, OX40L	Yes, Full length,	HEMDB62

7/16

Receptor Associated Factors

1, EB16 (TRAF1)

TRAF1(mouse)	Cell Aug. 26 1994
EB16 (human)	Nov. 28, 1994 (Submitted), Cell
<u>HNFD065 (HEMCL41)</u>	Feb. 10 1995 <u>Identified during Sept. 1994</u>

2, TRAP-3 (TRAF2)

TRAF2 (mouse)	Cell Aug. 26 1994
TRAP3 (human)	Mar 2 1995, deposited in GenBank, unpublished
<u>HMWCE69</u>	<u>Identified during Sept. 1994</u>

3, TRAF3 (CD40bp, LAP1, CRAF-1, CAP-1)

TRAF2 (mouse)	Cell Aug. 26 1994
CD40bp (human)	Sept. 22 1994 (submitted) JBC Dec. 2 1994
LAP1 (human)	Nov. 28, 1994 (Submitted), Cell Feb. 10 1995
CRAF-1 (human)	Science 10 1995
CAP-1 (human)	FEBS-Lett Jan 23 1995
<u>HTXFY28</u>	<u>Identified during Sept. 1994</u>

4, TRAP-1 Yes, Partial, HMEGG76

5, TRAP-2 Yes, Full length, HSLCL12

Receptors

1, TNFR1	Yes, Full length,	HSLAB61
2, TNFR2	Yes, Full length,	HMEAE09
3, TNFR-RP (LT β R)	Yes, Full length,	HOUEL78
4, Fas (APO)	Yes, Full length,	HSBAW14
5, CD40	Yes, Full length,	HCABR73
6, CD30	Yes, ,1280bp	HSEBA84
7, CD27	Yes, Full length,	HTTCB46
8, 4-1BB	Yes, Full length,	HTXER26
9, OX40	Yes, Full length,	HT5AI79
10, NGFR	Yes, -900bp	HCE1D55

Receptor Associated Factors

6, FADD (MORT1) Yes, Full length, HMEFX49, HUVCS86

FADD Feb. 23 1995 (Submitted) Cell May 19, 1995

MORT1 Jan 30 1995 (Submitted) JBC April 7, 1995

HUVCS86 Identified by death domain Jan 10 1995

7, RIP ??

8, TRADD Yes, Full, Splicing HT5AJ62, HT5AD11

9, FAP-1, Yes, Partial, HTEKR54

10, TRAK ??

11, FAST Yes, Full length, HODDG78

12, MLN62 Aug. 22 1995 (Deposited in GenBanK, unpublished)

HTXAF08 (HRGBO64) Identified on Nov. 1994

Novel TNF Ligand, Receptor and
Receptor Associated Factor Superfamily
Members in HGS Database

Ligands

I. Full length genes:

1, Fas ligand-like	HTPAN08
2, TNF-like (TNF gamma)	HUVEO91
3, TNF-like (TNF delta)	HLTBT71
4, TNF-like (TNF epsilon)	HPDD012

II. Genes are not full length

1, Lymphotoxin-beta-like	HTGAK40
2, Fas ligand-like	HYBAG72
3, Fas ligand-like	HEMBK77
4, Fas ligand-like	HBNAZ12
5, Lymphotoxin-like	HATAF70
6, Lymphotoxin-like	HE8CL19
7, CD30L-like	HKPAB54
8, CD30L-like	HHPFK35

Receptors

I. Full length genes

1, TNFR p75-like	HHFFT79
2, TNFR p75-like	HMSBZ59
3, TNFR p75-like	HT1SB52
4, TNFR p75-like	HSABH13

II. Genes are not full length

1, TNFR p55-like	HTTBN61
2, TNFR p75-like	HHFUB83
3, TNFR p55-like	HFVGO43
4, OX40-like	HRDBI28
5, OX40-like	HTXBO18
6, Fas-like	HCAAA38
7, CD40-like	HSIED66
8, CD40-like	HTXBS40
9, CD40-like	HELAO61
10, CD40-like	HETDB40
11, CD27-like	HCEFL28
12, CrmB-like	HDSAP50
13, 4-1BB-like	HOFMB09

Receptor Associated Factors

I. Full length genes:

1, TRAF2 (TRAP-3)	HMWCE69
2, TRAF2-like (MLN62)	HTXAF08X
3, TRADD splicing variant	HT5AJ62RA
4, EB16 (TRAF1)	HNFD065
5, TRAF3	HTXFY28
6, FADD	HUVCS86

II. Genes are not full length:

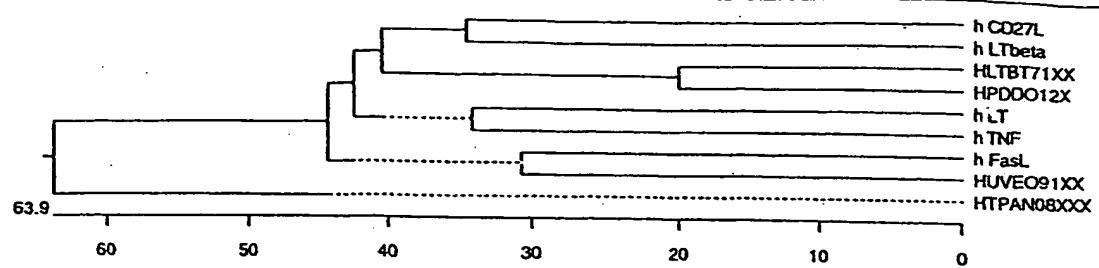
1, FADD-like	HHFHY89
2, TRAF-like	HE2PH69
3, TRAF-like	HETAP39
4, TRAF-like	HLMCT93
5, TRAF-like	HL2AH30
6, CD40bp-like	HUVCY67
7, TRAF-like	HLHDL86
8, TRAF-like	HLHCM30
9, TRAF-like	HJTAC77

- V A H - V I - P - Q - L L - - - W E - L R R G R A L L A S G V Majority									
	170	180	190	200					
69	- - - - -	Q D P R L Y	- - - - -	W Q G G P A L G R S F L	- H G P	h	C27L		
145	K V A H	- L T G R S N S R S H P E E	- - - - -	W E	- D T Y G I V L L S	- G Y	h	FasL	
63	P A A H	- L I G D F S K Q N - S D L	- - - - -	W R	- A N T D R R F B Q D G F	h	L T		
58	P A A H	- L I G A F	- E K G Q G L - G	W E	- T T K E Q A F L T S G	c	h	Lbeta	
66	P V A H	- W V A N P Q A E G - Q I Q	- - - - -	W L	- N F R A N A T L E N G V	h	TNF		
34	- - - - -	K N G P P A H	- - - - -	W E	- H E L G L A F T K N R K	h	HVE091XX		
133	- V T E - V H	- - - - -	- - - - -	W Q P A L R R G R G L Q A Q G Y	h	LT27T1XX			
52	- V T E - V H	- - - - -	- - - - -	W Q P A L R R G R G L Q A Q G Y	h	HPD0012X			
121	G R S N - T L S S E P N S K N E K A	E G R K I N S W E	- - - - -	S S R S G R S F L S N L	h	HPA008XXX			
E L S N G E - L V I P E A G L Y L V Y S Q V L F R G Q - - - C - - - S - - - Majority									
	210	220	230	240					
90	E I D K G Q - I R I H R D G I Y M U H I O N T L	- - - - -	A I C	- - - - -	S S T T	h	C27L		
175	K Y K E G G - I W I N R T G I F F V E S K Y F R E Q S	- - - - -	C	- - - - -	N N L P	h	FasL		
93	S I S S N S - N E V I T S G I F F V E S K Y F R E Q S	- - - - -	Y	- - - - -	S P K A	h	L T		
118	Q F S D A E G I A L L Q D G S V I Y L V C L E V G Y R R A P P G	- - - - -	G G D F	h	Lbeta				
118	E I R D H Q - I W V E S E Q G P I I V S P Q I V K E G G	- - - - -	C	- - - - -	P	h	TNF		
156	H Y T H K F - E L E R E S G D F I E S P Q I T F E G M T S E C	- - - - -	S E I R	h	HVE091XX				
154	- - - - - G V R I Q D A G V V L V E S A N D E Q D	- - - - -	- - - - -	- - - - -	-	h	LT27T1XX		
73	- - - - - G V R I Q D A G V V L V E S A N D E Q D	- - - - -	- - - - -	- - - - -	-	h	HPD0012X		
163	H G R R H G E - G G A V H E K G F S Y I S D O T Y E G F	- - - - -	E E	h	HPA008XXX				
- S R H V - L T H S M H G Q V V S K - G - G Y P E T L L E I S S K S S C P D R A Majority									
	250	260	270	280					
120	A S R H - - - H P T T L A V G I C S P A S R S I S I T R L S F H Q G C T I V S	- - - - -	h	C27L					
207	L S H K V Y M R N S - - - - - R - - - - -	W Q D L V V M E G R K M S V C T T G	h	FasL					
125	T S S P L Y M A H E V Q L F S S Q	- - - - - X P F V P D I S S Q R M V Y P	- - - - -	h	L T				
153	Q G R S V T K P S S L Y R A G G A Y G P G T P E L I D E G A E T V T P V L D P A	- - - - -	h	Lbeta					
147	- S T H U L M P H T I S R I A V S - - - - -	X Q T X V N I T S A I K S P C Q R E T	h	TNF					
90	Q A G R P N K E D S I T V W I T V T D S Y F P Q T Q L I M G T R S V C E V G S	- - - - -	h	HVE091XX					
174	- - - - - V R F T M Q D G V V R E G Q G R Q E M I F R C T R S M P S H B D R A	- - - - -	h	LT27T1XX					
93	- - - - - V R F T M Q D G V V R E G Q G R Q E M I F R C T R S M P S H B D R A	- - - - -	h	HPD0012X					
196	I K E N T K N D Q Q H V D Y I Y M Y T - S Y E D P I V I M K S A R N S C W S K D	- - - - -	h	HPA008XXX					
- W Y N S I Y L G G V F Q L T Q G D R L S V N I S R A S I V D - - Majority									
	290	300	310	320					
156	Q R - - - - -	L T P D A R G D T L C T H L T G - T I L P	- - - - -	h	C27L				
237	- - - - - Q M M A R S S P E G A V E N L S A D H L Y V A N V S E I S V N	- - - - -	h	FasL					
158	- - - - - G L Q E P H I L H S M H G A A F Q I E P Q D Q L S T H T D G I P H L V	- - - - -	h	L T					
193	R R Q G Y G P L H A T I S V G F G G L V Q I R R G E R V Y V N M S R P D M V D	- - - - -	h	Lbeta					
182	P E G A E A K P H S E P W A G G G F Q L E K G D R L S A E I N R P D Y L D	- - - - -	h	TNF					
130	N - - - - - E P Q P P Y D G A K F S L Q E G D K L M V N V S D I S I V D	- - - - -	h	HVE091XX					
208	- - - - - V N S C Y S C A G V F H L H Q E D I L E S V I P R A R A K L	- - - - -	h	LT27T1XX					
127	- - - - - V N S C Y S C A G V F H L H Q E D I L E S V I P R A R A K L	- - - - -	h	HPD0012X					
235	A E Y G L - - - - - Y S E M Q G G I F E L K E N D R I F V S V T N E H I D	- - - - -	h	HPA008XXX					

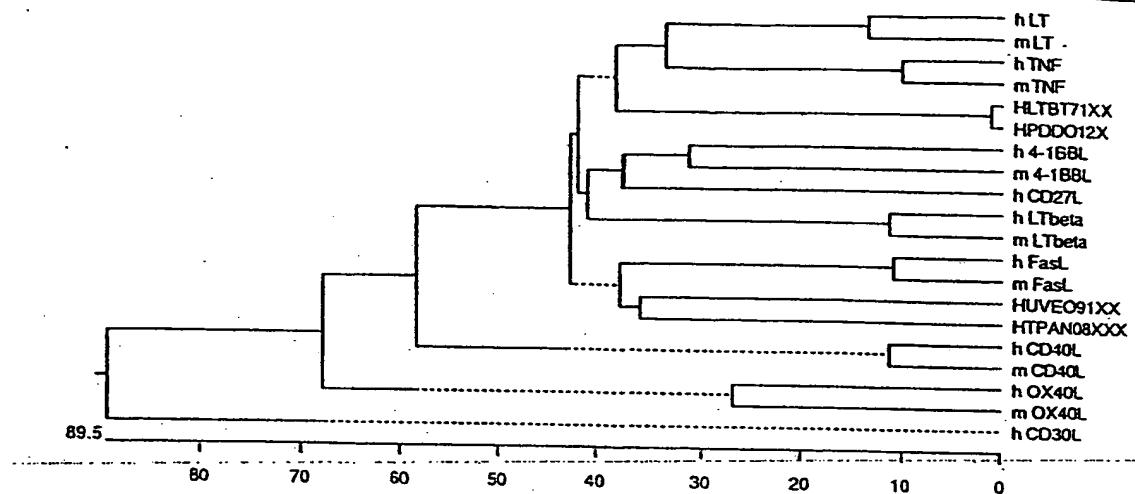
TM14

- F A S S C G T F F G A V K - V - L		Majority
330		
178	- S R N T D E T F E G V Q W - V R P	
269	- F E E S - Q D T E G C L Y R - - - L	h CD27L
193	- L S P S T - V F E G R F A - - - L	h FasL
231	- P A R - G K G E F G R V H - V G	h LT
220	- F G E S G Q V Y F G S I I A - - - L	h LTbeta
161	- Y T K E D K T F F K G A F L - L -	h TNF
237	- N L S P H G T S F L G F V R - L	GUVE091XX
156	- N L S P H G T F L G F V R - L	ELTBET71XX
268	- M D R E A S - F F G A F L - V G	EPDD0012X
		HTPAN08XXX

Decoration 'Decoration #1': Shade (with solid bright yellow) residues that match the Consensus exactly.

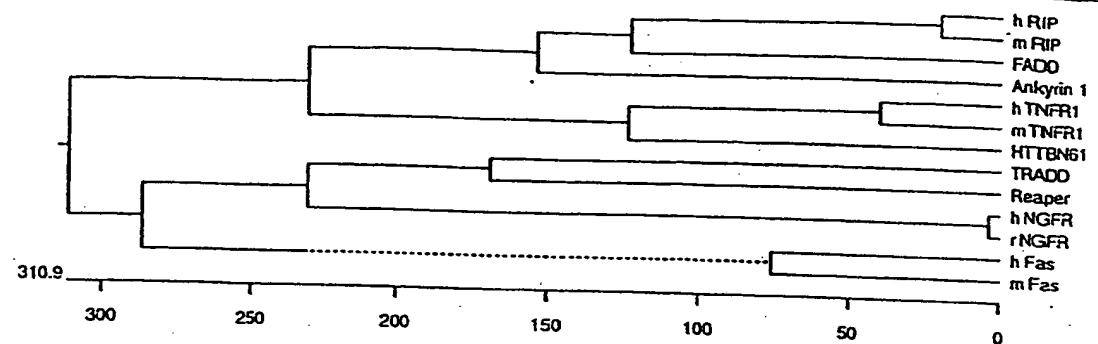


TN16

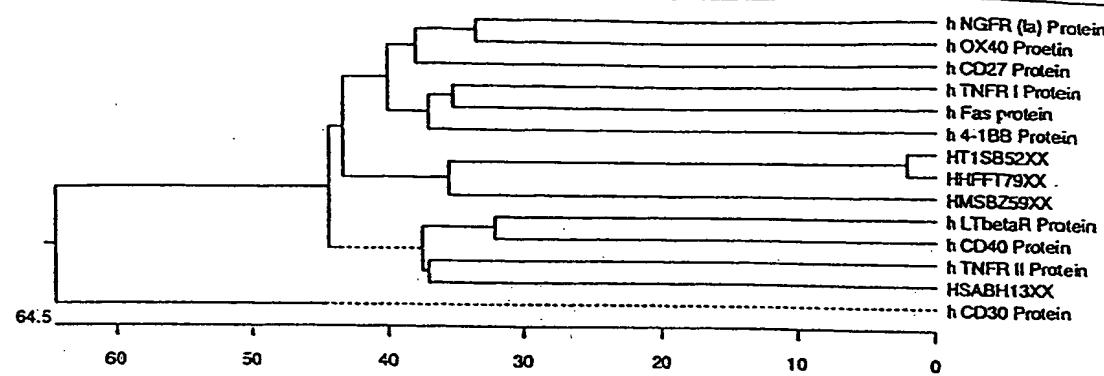


----- V I D E ----- G - W K E F A R E L ----- C L S E S E I Majority									
	10	20	30	40					
1	- N R P L S L K D Q Q T F A R S V G L K M R K V G R S L Q R G C R A E R D P A L	TRADD							
1	L C A A F N V I C D - - - - N V G Z D M R R L D R Q L - - - - K V S D T K M	FADD							
1	M A V A F Y I P D Q A T L L R E A E Q K E Q Q I L E - - - - - - - - - - - - - - - -	Reaper							
1	- -	Ankyrin 1							
1	I A G V M T L S Q V -	b Fas							
1	I A E D H T I Q E A -	m Fas							
1	- -	h RIP							
1	- -	m RIP							
1	- -	h TNFR1							
1	- -	m TNFR1							
1	- -	h NGFR							
1	- -	x NGFR							
1	- -	HTTEN61							
D S I E H E N G R D - L A E Q V Y Q L L R S W V Q R E G K K G A T L G K L - A A Majority									
	50	60	70	80					
40	D S L A Y E Y E R E G L Y E D O A P O L I R F R F V - - - Q A E G R A T L Q - - -	TRADD							
31	D S H E D D R Y P H E N - - - T E R V R E S S E R I N K N T E K E N - - - - - V A H E V G M	FADD							
33	R F L A T -	Reaper							
25	N R P R V E P P N S - - - D L D Q S T A L H T L S D V D R E G E N - - - E K H E N M Y T A	Ankyrin 1							
25	D E I K N D N V Q D T -	b Fas							
25	D E I M B D S I Q D T -	m Fas							
24	D E I D H D Y E E B D G -	h RIP							
24	D E I D H D Y E E B D G -	m RIP							
25	D R L E I L Q H G E C -	h TNFR1							
25	S R L E M O N G H C -	m TNFR1							
16	D S F T H S -	h NGFR							
16	D S F T H S -	m NGFR							
25	E A V E V E S I G H -	HTTEN61							
L R K I Q R - A D - L V N I L - Majority									
	90								
74	- -								
69	R S C G M - N L - V R D I V	TRADD							
61	- -	FADD							
63	L R N I D R - S E - I V M M E	Reaper							
63	L R E A N L C T L A - E K E Q T	Ankyrin 1							
63	L K R A E - C R R T H D K F Q D	b Fas							
64	L E Q C S R - I D - D L S S M	m Fas							
64	L H Q C C R - I D - D L N H E I	h RIP							
64	L R D M D L - L G C L E D D I E E	m TNFR1							
64	L I S K H M N L - R G C L E N T I E	m TNFR1							
47	L R R I Q R - A D - L V	h NGFR							
47	L R R I Q R - A D - I V	x NGFR							
60	W S A L	HTTEN61							

Decoration 'Decoration #1': Shade (with solid bright yellow) residues that match the Consensus exactly.



LLW - PL - LGL - L - LAS - PG - P - Majority



TNFR p55-like HTTB61

Query= Manual - 0
(1111 letters)

gp|M63122|PATTNFR_1 tumor necrosis factor receptor ... +1 107 8.0e-20 5
gp|X59238|XMP55R_1 p55 tumor necrosis factor receptor ... +1 106 2.8e-17 4
>gp|M63122|RATTNFR_1 tumor necrosis factor receptor (Rattus norvegicus)
>pir|S|GQRTT1 tumor necrosis factor receptor type 1 precursor - rat
Length = 461

Score = 107 (49.2 bits), Expect = 8.0e-20, Sum P(S) = 8.0e-20
Identities = 21/36 (58%), Positives = 26/36 (72%), Frame = +1

Query: 913 LYDVMMDAVPARRWKEFVPRTXGLRRAEETEAVEVEBGR 1020
LY V+D VP RWKEE+R GL E EIE +E++ GR
Sbjct: 365 LYAVVVGVPPTRMKEPMELALGLSSEHTEPLS1QNGR 401

Score = 79 (32.2 bits), Expect = 8.0e-20, Sum P(S) = 8.0e-20
Identities = 13/32 (40%), Positives = 18/32 (56%), Frame = +1

Query: 52 RGTIXLXAPCTEPCCRISTC1VCPQDTFLIGENH 147
+GT L + C P - C VC + TF A +NH
Sbjct: 54 KGTIVLVEDCPSGQETVCEVCDKGTFTASQNH 95

Score = 52 (26.7 bits), Expect = 2.0e-20, Sum P(S) = 2.0e-20
Identities = 11/32 (34%), Positives = 16/32 (50%), Frame = +1

Query: 157 AASPVNEQASQVALERCSAVADTRCGCIXGWF 262
- - - + QV + C A D C G C K F
Sbjct: 101 SCKTCRKEMPQVIEISPCKEADTVCGCKDQF 132

Score = 46 (21.2 bits), Expect = 8.0e-20, Sum P(S) = 8.0e-20
Identities = 7/16 (43%), Positives = 10/16 (62%), Frame = +1

Query: 1024 EDQQYEMEKRWPKHQ? 1071
R- Y MI+ WR + P
Sbjct: 404 REAHYSHLEAKPRRT? 419

Score = 39 (17.9 bits), Expect = 8.0e-20, Sum P(S) = 8.0e-20
Identities = 8/18 (44%), Positives = 10/18 (55%), Frame = +2

Query: 425 LPPCPAPRAPWQAVQSAV? 478
+P P? W V +A ?
Sbjct: 338 VPEPAPVRKWEDEVVAQ? 355

Score = 39 (17.9 bits), Expect = 4.5e-17, Sum P(S) = 4.5e-17
Identities = 6/17 (35%), Positives = 9/17 (52%), Frame = +2

Query: 215 CSAVADTRCGCIXGWFV 265
C +T C C G+E+
Sbjct: 158 CKEKQNTVCNCHAGFFL 174

Score = 38 (17.5 bits), Expect = 1.1e-05, Sum P(S) = 1.1e-05
Identities = 9/25 (36%), Positives = 10/25 (40%), Frame = +3

Query: 546 ANQHPEPTHTATAGLTSFWLQML 620
AN P H AT T L Y L
Sbjct: 413 ANRRRTPRHEATLDDVVGRLCDQML 437

TNFR p55-like HTTB61

Query= Manual - 0
(1111 letters)

g3 M59377 MUSTNFR2	Murine tumor necrosis factor II receptor (TNFR-2) mRNA, complete cds.	255	5.6e-12	2
g3 M60468 MUMTNFR1	Mouse tumor necrosis factor receptor ...	256	5.9e-12	2
g3 X59238 MCP55R	Murine mRNA for p55 tumor necrosis fa...	256	6.0e-12	2
g3 L26349 MUSTNFR	Mus musculus Tumor Necrosis Factor Re... ceptor	256	6.3e-12	2
g3 X57795 MUMTNFR5	Mouse mRNA for 55-kDa tumor necrosis ...	256	6.3e-12	2
g3 M60275 HUMTNFR2	Human tumor necrosis factor receptor(...	254	1.0e-11	1
g3 A21522 A21522	TNF alpha gene.	254	1.0e-11	1
g3 M33294 HUMTNFR	Human tumor necrosis factor receptor ...	254	1.0e-11	1
g3 M58286 HUMTNFR5	Homo sapiens tumor necrosis factor re... ceptor	254	1.0e-11	1
g3 M63121 HUMTNFR6C	Human tumor necrosis factor receptor ...	254	1.0e-11	1
>g3 M59377 MUSTNFR2	Murine tumor necrosis factor II receptor (TNFR-2) mRNA, complete cds. Length = 1956			

Plus Strand ESPs:

Score = 255 (71.7 bits), Expect = 5.6e-12, Sum P(2) = 5.6e-12
Identities = 78/112 (69%), Positives = 78/112 (69%), Strand = Plus / Plus

Query: 973 GGGCTGCGCGAGCCAGAGATCGAGCCGTGGAGGTGGAGATCGCCCGCTTCC 1024
||||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 1239 GGGCTGCGCGAGCCAGAGATCGAGCCGTGGAGATCGCCCGCTTCC 1281

Score = 63 (24.7 bits), Expect = 5.6e-12, Sum P(2) = 5.6e-12
Identities = 40/63 (52%), Positives = 40/63 (52%), Strand = Plus / Plus

Query: 194 TCCCACTGGCGCTGGAGAACTGTCAGGACTGGCCGACACCCGGCTGCGCTCTAAGASC 253
||||||| ||||| ||||| ||||| ||||| ||||| .
Subject: 423 TCCCACTGGAGACTGTCAGGACTGGCCGACACCCGGCTGCGCTCTAAGASC 423

Query: 254 GGCTGGTT 251
| |||
Sbjct: 463 AACCAAGTT 460

TNFR p55-like HFVGO43

>gp|M60468|MUSMTNF21_1 murine tumor necrosis factor receptor 1 [Mus musculus]
>

Score = 60 (27.2 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 14/35 (40%), Positives = 19/35 (54%), Frame = +3

Query: 15 VTXPKTPNHSPPSSPASQTXGFSKPSFLLSVXTEL 119

+T +P SP+S + T GFS P F V ST +

Sbjct: 264 LTPAPSPAFSPTSGFNPTLGFSTPGFSSPVSSTPI 298

Score = 37 (17.0 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 7/12 (58%), Positives = 9/12 (75%), Frame = +2

Query: 104 LLY*APCAVPXP 139

LLY + C+VP P

Sbjct: 325 LLYESLCSVVPAP 336

>gp|M76656|USTNFR20S3_1 tumor necrosis factor receptor [Mus musculus]
Length = 454

Score = 60 (27.2 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 14/35 (40%), Positives = 19/35 (54%), Frame = +3

Query: 15 VTXPKTPNHSPPSSPASQTXGFSKPSFLLSVXTEL 119

+T +P SP+S + T GFS P F V ST +

Sbjct: 264 LTPAPSPAFSPTSGFNPTLGFSTPGFSSPVSSTPI 298

Score = 37 (17.0 bits), Expect = 0.00066, Sum P(2) = 0.00066
Identities = 7/12 (58%), Positives = 9/12 (75%), Frame = +2

Query: 104 LLY*APCAVPXP 139

LLY + C+VP P

Sbjct: 325 LLYESLCSVVPAP 336

4-1BB-like

HOFMB09

>gb|U03397|HSU03397 Human receptor protein 4-1BB mRNA, complete cds.
Length = 1415

Plus Strand ESPs:

Score = 457 (129.0 bits), Expect = 1.5e-34, Sum P(2) = 1.5e-34
Identities = 129/174 (74%), Positives = 129/174 (74%), Strand = Plus / Plus

Query: 147 GCAAGATTCACCATGGCAGTGGCTATTACAACATAGTGCCGCTGTATTATGGTCAT 206
Sbjct: 147 GCGGCGCTGTATTATGGTCAT

5bjct: 167 CAACTTGGAGAGGACAGATCATTCAGGATCTTGTAGTAACGTGCCACGTGG 220

Score = 132 (35.5 Bits), Expect = 1.5e-34, Sum P(2) = 1.5e-34
Identities = 30/35 (85%), Positives = 30/35 (85%). Strand = Plus / Plus

Query:	19	AGTGGAAAGTTCTTACCCAGCCCTGANACCTCAAG	53
		1 1 1 1	
Sbjct:	1	AGTGGAAAGTTCTCCGGAGCCCTGAGATCTCAAG	35

>gp|003397|HSU003397_1 4-1BB [Homo sapiens]
Length = 255

Plus Strand HSPs:

Score = 138 (63.2 bits), Expect = 5.0e-20, Sum P(2) = 5.0e-20
Identities = 25/36 (69%), Positives = 29/36 (80%), Frame = +1

Query: 160 MGSGYYNIVAAVLLVMNPFERTRSIQDSCSKCPVGDF 257
MG+ YNIV +LLV+NFERTRS+QD CS CP G F
Sbjct: 1 MGNSCYNTIVATLLVIAFERTRSIQLDPCSNCPAGTE 35

Score = 73 (33.4 bits), Expect = 5.0e-20, Sum P(2) = 5.0e-20
Identities = 11/23 (47%), Positives = 15/23 (65%), Frame = +3

Query: 300 PCPPNFSNTSGQNXXSYARRCZ 368
PCPPN+FS+ GQ R+C+
Sbjct: 47 PCPPNFSNTSGQNXXSYARRCZ 368

TAN

CD40-like**HETDB40**

>gp|X75962|HSHOX40MR_1 OX40 homologue (Homo sapiens)
Length = 277
Score = 52 (23.9 bits), Expect = 0.026, Sum P(3) = 0.026
Identities = 9/17 (52%), Positives = 9/17 (52%), Frame = +2

Query: 200 PYGSYSNGSDCTRCPAG 250
P SY G DC CP G
Sbjct: 115 PLDSYKPGVDCAPCPPG 131
Score = 52 (23.9 bits), Expect = 0.036, Sum P(3) = 0.035
Identities = 10/38 (26%), Positives = 16/38 (42%), Frame = +3

Query: 786 LEASDVGSSCTSCPAGYYIDRDSGTCKSCPTNTILKAK 899
L++ G C CP G++ D+ C T+ H
Sbjct: 116 LD SYKPGVDCAPCPPGHS PGDNQACSEWTNCTLAGKH 153
Score = 51 (23.5 bits), Expect = 0.026, Sum P(3) = 0.026
Identities = 9/22 (40%), Positives = 11/22 (50%), Frame = +2

Query: 104 GAVKLLASGVKTHCPCNPGFF 169
G V + T C PC PGF+
Sbjct: 51 GMVSRCSRSQNTVCRPCGPGFY 72
Score = 45 (20.7 bits), Expect = 0.22, Sum P(3) = 0.20
Identities = 7/14 (50%), Positives = 9/14 (64%), Frame = +2

Query: 188 CQPCPYGSYSNGSD 229
C PCP G +S G +
Sbjct: 125 CAPCPGHS PGDN 138
Score = 44 (20.2 bits), Expect = 1.0, Sum P(3) = 0.64
Identities = 6/17 (35%), Positives = 12/17 (70%), Frame = +2

Query: 170 KTNNSTCQPCPYGSVSN 220
++ N+ C+PC G Y++
Sbjct: 58 RSQNTVCRPCGPGFYND 74
Score = 41 (18.9 bits), Expect = 0.026, Sum P(3) = 0.026
Identities = 9/22 (40%), Positives = 10/22 (45%), Frame = +3

Query: 615 PIPTSLRRTLPRASPGPSRGPL 680
PI PR S GPS P+
Sbjct: 186 PITVQPTEAWPRTSQGPSTRPV 207
Score = 40 (18.4 bits), Expect = 0.036, Sum P(3) = 0.035
Identities = 7/30 (23%), Positives = 16/30 (53%), Frame = +2

Query: 176 NNSTCQPCPYGSYSNGSDCTRCPAGTEAV 265
++ C+PC + + +GS+ + T+ V
Sbjct: 77 SS KPCPKPCWCNLRSGSERKQLCTATQDTV 106
Score = 36 (16.6 bits), Expect = 0.12, Sum P(3) = 0.12
Identities = 6/8 (75%), Positives = 7/8 (87%), Frame = +2

Query: 236 RCPAGTQP 259
RC AGT+P
Sbjct: 108 RCPAGTQP 115

Ligands

Cloning, Expression and Purification in Progress:

1, TNF-like (TNF delta) HLTBT71

Multiple tissue RNA blot analysis using HPDDO12 insert as probe showed that this gene is expressed in almost all the tissues tested. However, the expression in the heart was the highest followed by placenta and kidney.

2, TNF-like (TNF epsilon) HPDDO12

Receptors

I. HSABH13

TNFR p75-like

I, Protein was purified from baculovirus expression system.

II, Polyclonal antibody was made.

III, Functional study is in progress.

K.B. Tan
Alem Truneh
10.18.95

RNA Expression: TNF and TNFR Like Genes

TNFα (class 1)	TNFL1 413412 HTPAN03 ATG 343 pancreatic tumor (class 3)	TNFRL1 195197 HSABH13X ATG 348 fetal lung fibroblast line (class 2)	TNFRL2 103902 HTXBS40 ATG 363 T cell (class 2)	TNFRL3 231556 HTTBH61 ATG 358 testis tumor (class 5)	OX40 117992 HT4SD09 ATG 342 activated T cells (class 1)
--	--	--	--	--	--

SUMMARY OF RNA EXPRESSION STUDIES

Gene	Myeloid cells	B cell	T cell	Osteo-sarcoma	Tissues	Primary cells	Multiple forms
TNF α	HL60	REH (pre B)			Heart	CD4 $^+$	
TNFL1 pancreatic tumor	KG1a		Jurkat		Heart Bone marrow	CD4 $^+$ CD19 $^+$	Yes
TNFR1 fetal lung fibroblast				MG63	Kidney		
TNFR1.2 T cell	KG1a		Jurkat		Lung, Thymus Spleen Bone marrow	CD4 $^+$ CD8 $^+$ CD19 $^+$	
TNFR1.3 testis tumor	KG1a				Thymus	CD4 $^+$	Yes
OX40 activated T cell	KG1a		Jurkat			CD4 $^+$	

DR3

RNA Expression: TNF and TNFR Like Genes

DR 3

CELL LINES	TNF α (class 1)	TNFL1 413412 Pancreatic tumor (class 3)	TNFR1 195197 Lung fibroblast (class 2)	TNFR2 103902 T cell (class 2)	TNFR3 231556 Testis tumor (class 5)	OX40 117992 Activated T cells (class 1)
<i>Bone Marrow Stromal</i>						
TF274	-	\pm	+	-	-	-
<i>Osteosarcoma</i>						
MG63	-	-	++	-	-	-
HOS (TE85)	\pm	\pm	-	-	-	-
<i>Hematopoietic: Erythroid</i>						
K562			-			
<i>Hematopoietic: Myeloid</i>						
KG1a	-	++	-	+	++	+
KG1	-	++	-	\pm	+	\pm
PLB985			-			
HL60	++	-	++	-	-	-
U937			-	\pm	-	-
THP-1	\pm	\pm	\pm	+	-	-
<i>Hematopoietic: B-Lymphocyte</i>						
REH (Pre B)	+	-	-	\pm	-	-
BJAB (IgM)			-	-	-	-
Raji (IgM)	\pm	-	-	\pm	-	-
IM-9 (IgG)			-	-	-	-
<i>Hematopoietic: T-Lymphocyte</i>						
Sup-T1			-	-	-	-
Jurkat	-	+	-	+	-	+
H9				+	-	-
Molt-3	-	-	-	\pm	-	-
<i>Endometrial Carcinoma</i>						
RL95-2			-	-	-	-
<i>Breast Cancer</i>						
MCF7	-	-	\pm	-	-	-
<i>Colon Cancer</i>						
BE			-	-	-	-
HT29			-	-	-	-
<i>Neuroblastoma</i>						
IMR32				-	-	-

no entry = not tested, - = not detected, \pm to ++ = increasing amounts of RNA detected.

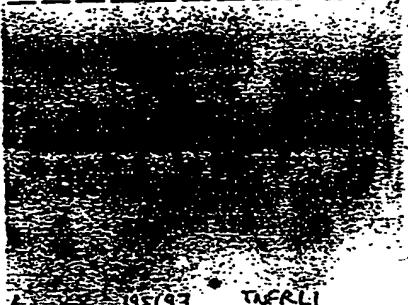
L02

RNA Expression: TNF and TNFR Like Genes DR 3

TISSUES and CELLS	TNF α (class 1)	TNFL1 413412 HTPAN08 ATG 343 pancreatic tumor (class 3)	TNFRL1 195197 HSABH13X ATG 348 fetal lung fibroblast line (class 2)	TNFRL2 103902 HTXBS40 ATG 363 T cell (class 2)	TNFRL3 231556 HTTBH61 ATG 338 testis tumor (class 5)	OX40 117992 HT4SD09 ATG 342 activated T cells (class 1)
HUMAN						
Brain	-	-	-	-	-	-
Heart	++	++	+	+	-	-
Lung	\pm	+	+	++	\pm	-
Thymus	-	-	-	++	-	-
Spleen	\pm	\pm	-	++	++	-
Liver	-	-	-	-	-	-
Kidney	-	+	++	+	-	-
Small Intestine	\pm	-	-	+	\pm	-
Prostate	-	+	-	+	-	-
Skeletal Muscle	\pm	-	-	-	-	-
Bone Marrow	\pm	++	-	++	\pm	-
PBL, CD19+	\pm	++	-	++	\pm	-
PBL, CD8+	\pm	+	-	++	-	-
PBL, CD4+ (activated)	+++	+++	\pm	++	++	++
RAT						
Brain	-	-	-	-	-	-
Heart	++	-	-	-	-	-
Lung	-	-	-	-	-	-
Thymus	-	-	-	-	-	-
Spleen	-	-	-	-	-	-
Kidney	+	-	-	-	-	-
Small Intestine	-	-	-	-	-	-

- = not detected, \pm to +++ = increasing amounts of RNA detected.

B H W T S L K E P M Juket



105/02 TNFLI

B H W T S L K I P M Juket



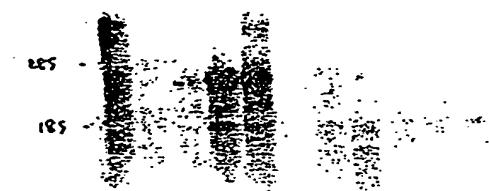
5.363 403982 TNFLI 2

Barin Heart Lung Stomach Spleen Liver Kidney Testis Prostate Bladder

Juket

2-18-65(C) TAC
01444660

B H W T S L K I P M Juket



185

5.338 231556 TNFLI 3

B H W T S L K I P M Juket



5.343 413412 TNFLI

1.05

9-24-95 0240

meat
Trotter
Salad
ice
Hus
Tuna
egg
cater
beefs
meat
meat

ME63
TFS14
L416
KG1
H10
THP1
CD19⁺
CD4⁺
B1605
ME57
Molt 3

44-1566 446365-102902

9.6. (16) THE W

6663	7804	Kala	Kel	Maw	THEI	GANT	gat	Amor	met	Moh
------	------	------	-----	-----	------	------	-----	------	-----	-----

ME681 TP214 XG14 K51 14661 THP1 CDR1 C941 00105 M67 molts

424561 426338 THERESA

9-20-98 (L) 4TG 143 TNFLG13412-

PAGE 55

Raji	Raji/Hu	REH	CD8 ⁺	BM	Thymus	RAT
					spleen	
					Heart	
					Lung	
					Kidney	
					S. Sulfathiazine	

TNFRL3

Raji	Raji/Hu	REH	CD8 ⁺	BM	Thymus	RAT
					spleen	
					Heart	
					Lung	
					Kidney	
					S. Sulfathiazine	

TNFRL1

Raji	Raji/Hu	REH	CD8 ⁺	BM	Thymus	RAT
					spleen	
					Heart	
					Lung	
					Kidney	
					S. Sulfathiazine	

TNFRL4

Raji	Raji/Hu	REH	CD8 ⁺	BM	Thymus	RAT
					spleen	
					Heart	
					Lung	
					Kidney	
					S. Sulfathiazine	

OX40

TNFRL2

Raji	Raji/Hu	REH	CD8 ⁺	BM	Thymus	RAT
					spleen	
					Heart	
					Lung	
					Kidney	
					S. Sulfathiazine	

TNFRC

LOZ

TNFRL2-Ig Binding to Cells

	Cell Line	Cell Lineage	Unstained	GAH-FITC Control	TNFRL2-Ig Binding
1)	THP1	Monocyte	2.2	5.2	51.0
	THP1/ LPS		2.8	5.4	53.1
2)	HL60	Pro-monocyte	2.0	5.2	138.3
	HL60 / PHA+PMA		2.4	10.6	171.8
3)	H9	T	2.1	6.3	14.3
	H9 / PHA+PMA		3.9	23.1	43.8
4)	Jurkat	T	2.1	3.6	19.4
	Jurkat / PHA+PMA		2.4	3.7	12.4
5)	KG1a	Myeloid Precursor	2.6	6.4	12.3
	KG1a / PHA+PMA		2.8	5.6	19.9

EXPRESSION OF TNF-LIKE PROTEIN

HGS clone 413412 = HTPAN08

Objectives:

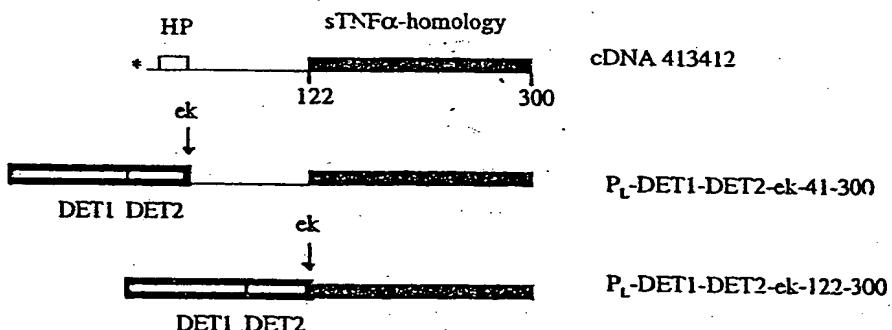
- Express as fusion protein in *E. coli*—to be used for raising antibodies
- Express in soluble form in *E. coli* or other systems—to be used for receptor binding and activity assays

Accomplishments:

- Plasmid DNA from HGS strain (purified by K.-B. Tan) was sequenced.
The DNA sequence in the open reading frame agreed with that reported by HGS.

There was a 1 bp difference upstream of the first of 3 closely spaced in-frame ATG codons, which makes it more likely that the first ATG is the translation initiator codon (TTCATGG in HGS sequence is ATCATGG in SB sequence; the latter is in good agreement with Kozak consensus upstream sequence)

- Two fusion constructs were made for *E. coli* expression; both expressed well as insoluble proteins; both have the potential for release of untagged protein following enterokinase digestion. Protein purification/rabbit injection is planned.



HP=hydrophobic
DET1=epitope gp120
DET2=hexaHis
ek=enterokinase site
P_L=promoter
* = 3 ATG codons
(codons numbered from 1st ATG)

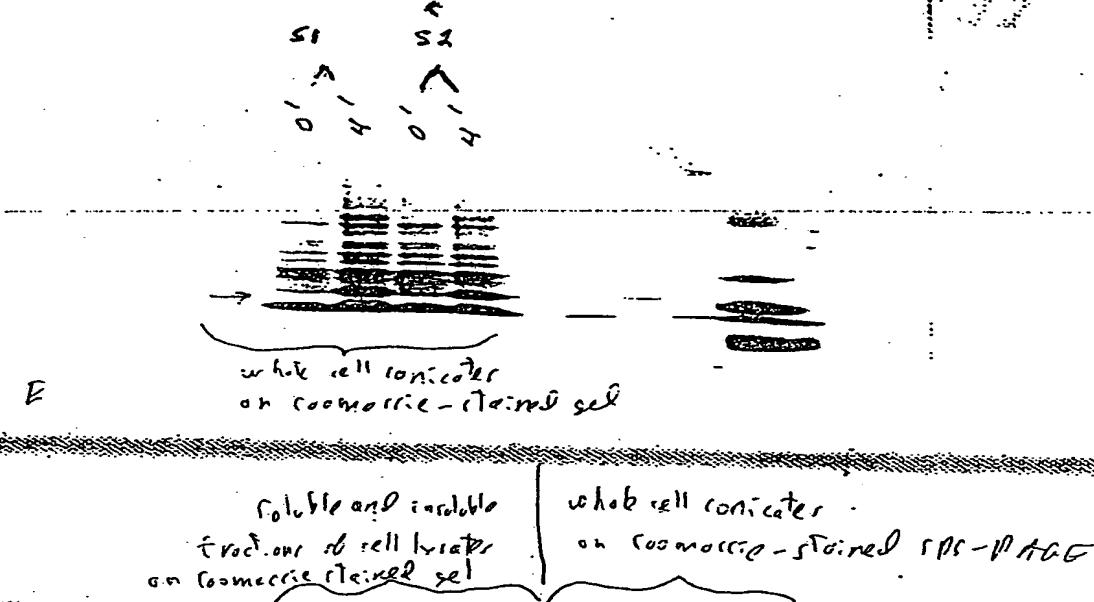
FAI

UV1

Expression of clone 413412 fusions
in E. coli:

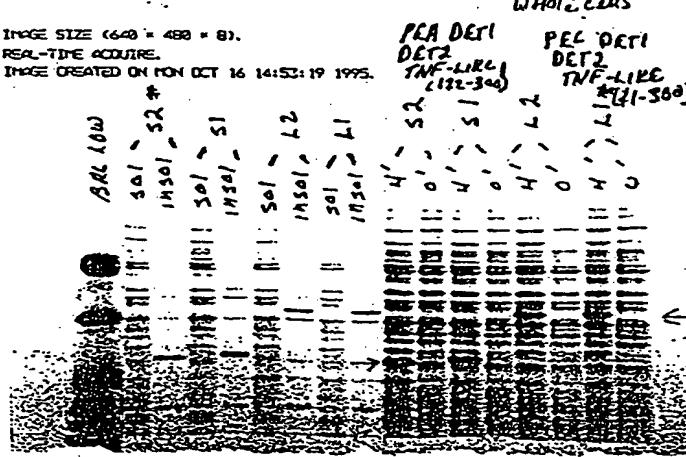
STRATEGENE EAGLE EYE II 10/16/95 14:59:23

IMAGE SIZE (640 x 480 x 8).
REAL-TIME ACQUIRE.
IMAGE CREATED ON MON OCT 16 14:59:21 1995.



STRATEGENE EAGLE EYE II 10/16/95 14:53:22

IMAGE SIZE (640 x 480 x 8).
REAL-TIME ACQUIRE.
IMAGE CREATED ON MON OCT 16 14:53:19 1995.

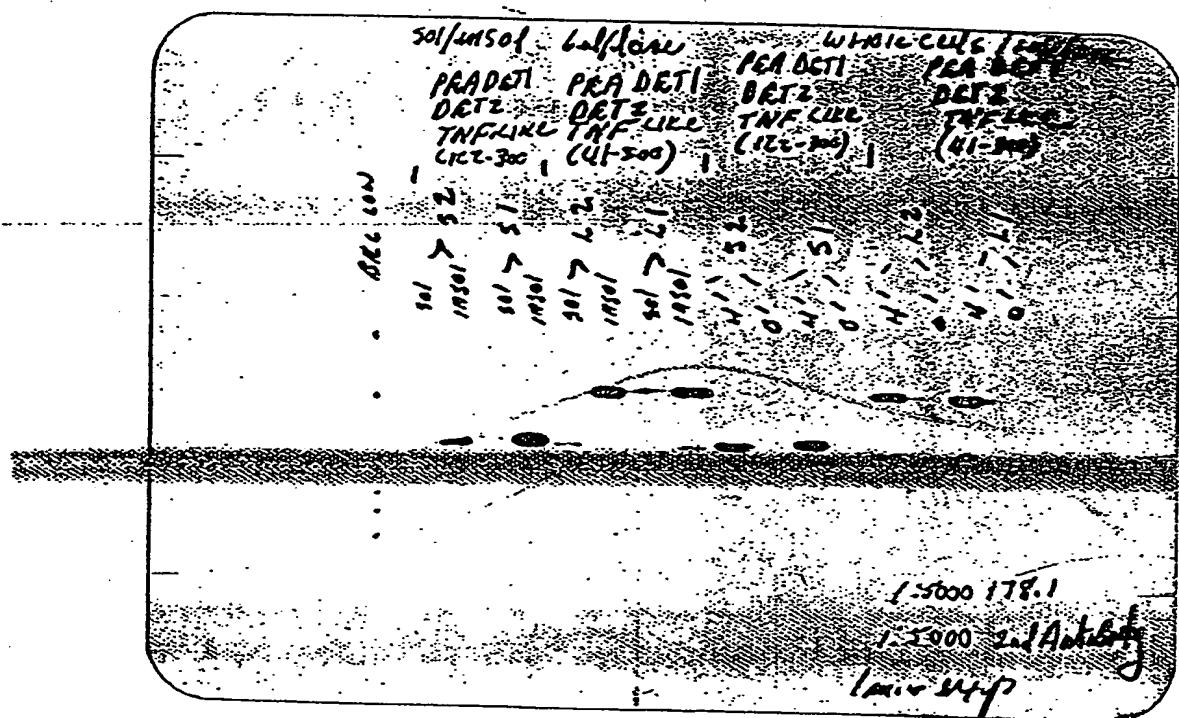


Duf

Expression of clone 413412 fusion
in E. coli -

10/20/2015 15:15

Western blot with anti-DE71 Mab



FASTA of HSAV045 vs Death Domain Proteins

The best scores are:

			init1	initn	opt
Sw:Tnr1_Human	P19438	homo sapiens (human).	tumor necrosi...	111	153
Sw:Tnr1_Mouse	P25118	mus musculus (mouse).	tumor necrosi...	113	144
Sw:Ank1_Human	P16157	homo sapiens (human).	ankyrin r (can...	47	47
Com\$94:[Hurlemr.Sequences.Pep]Rip.Pep		mouse RIP		36	36
Sw:Fasa_Human	P25445	homo sapiens (human).	fasl receptor...	29	29
Sw:Ngfr_Human	P08138	homo sapiens (human).	low-affinity ...	28	28
Sw:Ngfr_Rat	P07174	rattus norvegicus (rat).	low-affinity...	28	28
Sw:Ngfr_Chick	P18519	gallus gallus (chicken).	low-affini...	28	28
Com\$94:[Hurlemr.Sequences.Pep]Fadd.Pep		FADD/MORT1		27	27
Sw:Ankc_Human	Q01485	homo sapiens (human).	ankyrin, brai...	26	26
Com\$94:[Hurlemr.Sequences.Pep]Tradd.Pep		TRADD		24	24
Sw:Fasa_Mouse	P25446	mus musculus (mouse).	fasl receptor...	22	22

	PR1	R1	R2	I1	R3	I3	R4	R5	I2	R6	I4	R7	PR7
IHLHAB49	1	X	X	X	X	X	X	X	X	X	X	X	(full length)
IHT0AU65		X	X	X	X	X	X	X	X	X	X	X	(full length)
IHT1SB52	3	?	X	X	X	X	X	X	X	X	X	X	(full length)
IHT0BH42	3	?	X	X	X	X	X	X	X	X	X	X	(full length)
IHLHTJ50	1	X	X	X	X	X	X	X	X	X	X	X	(full length)
IHLMCG62		X	X										
IHT0HT08		X	X										
IHTXBS40			X										
IHTXFP69				X									
IHTXE024					X								
IHLMAC26						X							
IHMQCG18							X						
IHEAAQ14								X					
IHCUBW34									X				
IHBNAG20										X			
IHOABR18											X		

X (discontinuous ESTs)

	PR1	R1	R2	I1	R3	I3	R4	R5	I2	R6	I4	R7	PR7
IHHFFET79	1	X	X	X	X	X	X	X	X	X	X	X	(full length)
IHBNAT51													
IHT4SI80													

	PR1	R1	R2	I1	R3	I3	R4	R5	I2	R6	I4	R7	PR7
IHMSBZ59		X	X	X	X	X	X	X	X	X	X	X	(full length)
IHFNB43													

	PR1	R1	R2	I1	R3	I3	R4	R5	I2	R6	I4	R7	PR7
IHROAG75	2	X											

mt/s

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